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# Method for Stabilization and Improvement of Gene Transfer into Mammalian Cells

#### **Description**

The invention refers to a method for stabilization and improvement of gene transfer into mammalian cells which is characterized by overexpression of the gene of the cell cycle regulator p21<sup>WAF1/CIP1</sup> (p21) an inhibitor of cyclin-dependent kinases (CDK).

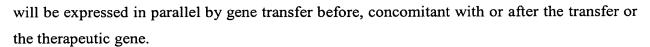
By the use of already known techniques, genes or their cDNA's are transported into target cells in order to express the corresponding protein product. To do so, there exist different methods like transfer of naked DNA or by the help of viral and/or non-viral transfer systems (vector). These transfer systems contain an expression cassette which carries the gene or its cDNA and, if necessary, a promoter into a target cell. Depending on the final goal, there is a great variety of methods to transfer a gene of choice into a given target cell. For the expression of the transgene the host cell is used to transform and translate the genetic information into the corresponding protein.

One limitation of the efficacy of such gene transfer techniques is the induction of programmed cell death (apoptosis) which, in turn, is dependent on different factors like type and dose of the vector and/or expression cassette construct used.

Immunological effects limit the efficacy of gene transfer in vivo like e.g. in adenovirusmediated gene transfer. However, induction of apoptosis is one of the main mechanism that limits both, in vivo and in vitro efficacy.

Therefore, the underlying concept of the invention was to develop a method which can prevent the induction of apoptosis in a target cell during and/or after gene transfer and, consequently, improves the gene transfer into and/or prolongs the expression in a mammalian cell of choice.

The task is completed by the transfer of genetic material like a therapeutic gene which represents the DNA and/or cDNA of a gene that is defective and/or deleted in the disease to be treated and that the therapeutic gene is transferred by known gene transfer techniques, if necessary in conjunction with a promoter, and that after the transfer the therapeutic gene is expressed in the target cell. In agreement to the claims, a cDNA of the cell cycle regulator p21



P21 is a known cell cycle regulator which prevents re-entry of senescent cells into cell cycle progression by blockage of cyclin-dependent kinases. This function includes different mechanisms like hypophosphorylation of the protein product of the Retinoblastoma Gene (Rb), binding to proliferating cell nuclear antigen (PCNA), binding to CDK-cyclin complexes like cyclin D-CDK4, cyclin E-CDK2, and cyclin A-CDK2. Whereas the interaction between p21 and PCNA prevents DNA replication, the interaction of p21 with cyclin dependent kinase complexes results in arrest of the cell cycle in the G<sub>1</sub>-phase. The presence of p21 and of its cellular function is of vital importance for the survival of a cell. This importance is, for instance, illustrated by the fact, that there exist almost no mutations that are able to survive.

The invented method surprisingly prevents the induction of apoptosis of a target cell after gene transfer by overexpression of p21.

As known, eukaryotic cells replicate their genome only during a defined and limited period of time which is termed as phase of DNA synthesis (S-Phase) of the cell cycle. The cell cycle comprises four phases: G<sub>1</sub>-phase, S-Phase, G<sub>2</sub>-phase and Mitosis. The duration of each phase is rather constant. The G<sub>1</sub>-phase lasts in fast proliferating cells between 2 and 20 hours, S-Phase between 6 and 10 hours, G<sub>2</sub>-phase between 2 and 4 hours and Mitosis between 3 and 4 hours.

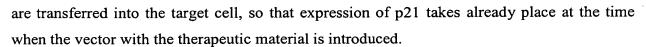
For gene transfer already known techniques are used, where the gene of choice can be transferred as naked DNA or by packaging it in different types of vectors which can be of non-viral or viral nature. Preferentially, viral vectors are used like adenovirus, retrovirus, baculovirus, parvovirus and/or herpes virus or others.

In correspondence to the invention, one application is performed by placing the cDNA of a given therapeutic gene and the cDNA of p21 in the same gene transfer vector.

Afterwards, the vector system is transferred into the target cell were the protein of choice and p21 are expressed.

Another preferred application is characterized by the fact that the cDNA of a given therapeutic gene and the cDNA of p21 are localized on different gene transfer vector. The transfection into the target cell takes place at the same time.

In another application, the vector which carries the material for the expression of p21 can be introduced into the target cell first. Afterward the vector which carries the transgene of choice



Moreover, the invention encompasses also a viral vector system for the optimization of the gene transfer which is characterized by

- the nucleic acid of a viral vector containing the genetic material to be transferred and that of p21
- the nucleic acid of a viral vector containing the genetic material and the nucleic acid of a viral vector containing the genetic material of p21

Especially, adenovirus based gene transfer vectors are preferred. Therefore the invention will be explained in more detail using an example of adenovirus based gene transfer vectors.

One of the most commonly used vectors systems to transfer genetic material are adenovirus vectors (Ad vector). Because of their high titer and their stability in blood, the Ad vector is especially efficient for in vitro and in vivo gene transfer. Natural target cells for the adenovirus are epithelial based cells and tissue. After infection of replicating and/or non-replicating cells, the adenoviral genome is located in the nucleus in an epichromosomal fashion resulting in a temporary gene expression.

Fig. 1 demonstrates apoptosis of Ad vector infected cells exemplified by the use of an Ad vector which carries the gene for human alpha-1 antitrypsin. This takes place by activation of S-phase without subsequent mitosis leading to deformed polyploid nuclei.

In agreement with the claims, the Ad vector induced apoptosis of the target cells could be prevented by ectopic overexpression, i.e. co-expression, of the cell cycle regulator p21. Using the same type of Ad vector as in Fig. 1, the infected target cells survived and were free of deformed polyploid nuclei and no apoptosis occurred after adenovirus-mediated gene transfer of p21 (Fig. 2).

That p21 can indeed prevent Ad vector mediated apoptosis of the infected target cell can be seen in Fig. 3 which demonstrates that S phase activation can be prevented by adenovirus-mediated overexpression of p21.

#### Figure Legends

#### Figure 1

Replication-deficient recombinant adenovirus vector induces apoptosis by uncoupling of S-phase and mitosis. The corresponding flow cytometry analysis of cell cycle distribution (A, B) and in situ detection of apoptosis by TUNBL-assay (C, D) are demonstrated for LoVo cells 48 h after Ad vector infection. Cells were mock infected (buffer control) (A, C) or infected with an Ad vector carrying alpha-1 antitrypsin (100 plaque forming units per cell) (B, D).

### Figure 2

Overexpression of p21 prevents adenovirus-induced apoptosis. In situ detection of apoptosis in LoVo cells 48 h after infection. Cells were mock infected with buffer (A, D), infected with an Ad vector carrying the human alpha-1 antitrypsin at a dose of 100 plaque forming units per cell (B, E) or infected with an Ad vector coding for p21 (100 plaque forming units per cell) (C, F). Shown are representative photographs at a magnification of 200-fold (A to C) and 600-fold (D to F).

## Figure 3

P21 protects against adenovirus-mediated apoptosis by prevention of a G2-like arrest. Demonstrated is the flow cytometry analysis of cell cycle distribution of LoVo cells after 48 h of infection with different doses of Ad vectors expressing either the cDNA of human alpha-1 antitrypsin ( ) or the cDNA of p21 ( ). Shown are the relative percentages of the cell populations which are in the  $G_0/G_1$  or  $G_2/M$  phase of the cell cycle as well as the percentage of living cells in the whole population (negative in the propidiumiodid staining; PI). The data represent the mean  $\pm$  standard error of three experiments.

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